

CATECHOLAMINES

Chromatographic – Fluorimetric Determination of Free, Free+Conjugated, Total (Epinephrine + Norepinephrine) or Fractionated (Epinephrine / Norepinephrine) Catecholamines in Urine

40 tests

REF 3630

INTENDED USE

Kit for quantitative *in vitro* determination of Free, Free+ Conjugated, Total (Epinephrine + Norepinephrine) or Fractionated (Epinephrine/norepinephrine) catecholamines in urine.

ASSAY PRINCIPLE

Catecholamines are adsorbed on an cationic buffer balanced resin. After interfering substances washing, catecholamines are fluorometrically defined by oxidation with ferricyanide and transformation into trihydroxyindoles with hydrated sodium.

REAGENTS AND COLUMNS

Kit components:

REF 3630

*REAGENT 1 Blue bromthymol	1 x 5 ml
*REAGENT 2 Hydrated sodium	1 x 60 ml
REAGENT 3 Phosphate buffer	2 x 210 ml
REAGENT 4 Boric acid	2 x 220 ml
WARNING: before use, re-dissolve any eventual precipitate on the bottom of the vial, by warming in a light bain-marie.	
REAGENT 5 Phosphate buffer	1 x 60 ml
WARNING: same as Reagent 4.	
REAGENT 6 Sulphate zinc	1 x 15 ml
REAGENT 7 Potassium ferricyanide	1 x 15 ml
REAGENT 8/A Ascorbic acid	5 vials
*REAGENT 8/B Hydrated sodium	1 x 160 ml
REAGENT 9 Norepinephrine standard 100 mg/L	1 x 3 ml
REAGENT 10 Epinephrine standard 100 mg/L	1 x 3 ml
COLUMNS Chromatographic columns	40

(* Dangerous reagents are marked by an asterisk. Refer to MSDS.

STABILITY: stored at 2-8°C, sealed reagents and columns are stable up to the expiry date on the label.

EQUIPMENT REQUIRED BUT NOT SUPPLIED

– Spectrofluorometer or filter fluorometer:
Excitation: 405 nm Emission: 495 nm
436 nm 540 nm

PREPARATION OF WORKING REAGENTS

REAGENT 8/A

Dissolve the contents of a vial of Reagent 8/A with 5 ml of distilled water. Shake gently until complete dissolution.
STABILITY: at least 5 days at 2-8°C.

REAGENT 8 (8/A + 8/B)

Add 0.3 ml of dissolved Reagent 8/A to 10 ml of Reagent 8/B and mix to obtain an homogeneous solution.

STABILITY: prepare Reagent 8 immediately before the test and use it within 20 minutes.

REAGENTS 9 and 10 (standard diluted epinephrine and norepinephrine)

Dilute Reagents 9 and 10 in volumetric ratio 1:1000 with Reagent 4 (example: 20 µl of Reagents 9 or 10 carried to 20 ml with Reagent 4).

STABILITY: at least 5 days at 2-8°C, if protected from light. Any eventual precipitate does not interfere with the reagent performance.

SAMPLE

24 hour urine. Collect the 24 hour urine in a polyethylene container with 15 ml hydrochloric acid 6M (concentrated hydrochloric acid diluted 1:2 with distilled water) and adjust the pH to approximately 3. Mix, measure the volume and store at 2-8°C. Centrifuge or filter the urine before use.

STABILITY: at least 3 months at 2-8°C, protected from light.

Determination of total catecholamines (free + conjugated): pipette 5 ml of sample into a test-tube and adjust the pH up to 0.5-0.9 with hydrochloric acid 6M. Put the test-tube into a hot bain-marie for 20 minutes. Cool under running water and proceed as for the free catecholamines test.

MANUAL ASSAY PROCEDURE

Wavelength:

total catecholamines (epinephrine+norepinephrine):

excitation	405 nm	Turner	110-812
emission	495 nm	Turner	110-825 (65 A)

fractionated catecholamines (epinephrine/norepinephrine):

excitation	405 nm	Turner	110-812
	436 nm	Turner	110-916 (2A)+110-812 (47B)
emission	495 nm	Turner	110-825 (65 A)
	540 nm	Turner	110-822 (58) + 110-826 (2A - 15)

Temperature:	room temperature
Linearity:	up to 5 mg/L
Sensitivity:	10 µg/L
Recovery:	90±5 %
C.V.:	2%

PREPARATION OF THE SAMPLE

Pipette into a test-tube:

Urine	5 ml
Reagent 1	1 drop

Mix thoroughly and add:

Reagent 2	drop by drop, mixing until the solution turns into blue-green (pH 6.5), in doubt use a ph-meter
Reagent 3	10 ml

Mix well.

PREPARATION OF THE COLUMN

Mix and turn the columns upside down to obtain a complete resin resuspension. Then leave the columns for a few minutes in vertical position to allow the resin to sediment again. Take the upper cap off and snap the bottom tip off. Let the liquid completely flow.

CHROMATOGRAPHIC SEPARATION

Pour the test-tube contents into one column in several times and let it completely drain. Discard the eluate.

Wash each test-tube with 10 ml of distilled water, pour into the column and let the liquid completely drain. Discard the eluate.

Pipette into the column:

Distilled water	10.0 ml	discard the eluate
Reagent 4	7.5 ml	collect the eluate

Mix the collected eluate.

WARNING: at this step, the test can be interrupted for 24 hours maximum, storing the eluate at 2-8°C into a sealed test-tube, protected from light.

FLUOROMETRY

Label some test-tubes of 15 ml as it follows:

S: Sample, B/S: Blank Sample, N/St: Norepinephrine Standard, N/B/St (Norepinephrine Blank Standard) and, in case of fractionated catecholamines determination, E/St (Epinephrine Standard), E/B/St (Epinephrine Blank Standard)

Pipette into the test-tubes the following volumes in ml:

	S	B/S	N/St	N/B/St	E/St	E/B/St
Eluate	1.75	1.75	---	---	---	---
Diluted Reagent 9	---	---	1.75	1.75	---	---
Diluted Reagent 10	---	---	---	---	1.75	1.75
Reagent 5	0.5	0.5	0.5	0.5	0.5	0.5
Reagent 6	0.1	0.1	0.1	0.1	0.1	0.1
Reagent 8	---	0.5	---	0.5	---	0.5

Shake accurately.

At regular time intervals (for example: 15 seconds; with this interval 8 samples maximum can be fluorometrically determined at a time), add and mix thoroughly after the reagent is added:

Reagent 7	0.1	---	0.1	---	0.1	---
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Wait for exactly 2 minutes. At same time and sequence intervals used for Reagent 7 addition, add and mix accurately:

	S	B/S	N/St	N/B/St	E/St	E/B/St
Reagent 8	0.5	---	0.5	---	0.5	---
Reagent 7	---	0.1	---	0.1	---	0.1

After 10 minutes, pour into fluorimetric cuvettes and within 20 minutes read the fluorescences of the samples, the standards and the blanks at the following wavelengths: Excitation: 405 nm, Emission: 495 nm.

For the fractionated catecholamine determination, read also at the following wavelengths: Excitation: 436 nm Emission: 540 nm.

CALCULATION

TOTAL CATECHOLAMINES (epinephrine + norepinephrine):
readings at 405-495 nm

Total catecholamines, expressed as norepinephrine

$$(\mu\text{g}/100 \text{ ml}) = [(S - B/S) / (N/St - N/B/St)] \times 15$$

$$\text{Total catecholamines } (\mu\text{g}/24 \text{ hour}) = \mu\text{g total catecholamines}/100 \text{ ml urine} \times 10 \times L \text{ 24 hour/ urine}$$

FRACTIONATED CATECHOLAMINES (epinephrine + norepinephrine):

Calculate the following clear fluorescences where indicated:

(a) fluorescences at 405/495 nm

(b) fluorescences at 436/540 nm

$$S1 = S_a - B/S_a \quad N1 = N/St_a - N/B/St_a \quad E1 = E/St_a - E/B/St_a$$

$$S2 = S_b - B/S_b \quad N2 = N/St_b - N/B/St_b \quad E2 = E/St_b - E/B/St_b$$

$$\frac{(S1 \times E2) - (S2 \times E1)}{(N1 \times E2) - (N2 \times E1)} = Y$$

$$(N1 \times E2) - (N2 \times E1)$$

$$\text{Norepinephrine } (\mu\text{g}/100 \text{ ml}) = 15 \times Y$$

$$\text{Epinephrine } (\mu\text{g}/100 \text{ ml}) = 15 \times [S2 - (Y \times N2)] / E2$$

$$\mu\text{g}/24 \text{ hour} = \mu\text{g}/100 \text{ ml} \times 10 \times L \text{ 24 hour urine}$$

REFERENCE VALUES

Total free catecholamines (epinephrine + norepinephrine):

Children: 0.4 - 2.0 $\mu\text{g}/\text{kg}/24 \text{ hours}$

Adults: 1 - 13 $\mu\text{g}/100 \text{ ml}$ (10 - 100 $\mu\text{g}/24 \text{ hours}$)

Fractionated free catecholamines (epinephrine): 10 \pm 10 $\mu\text{g}/24 \text{ hours}$

Fractionated free catecholamines (norepinephrine): 40 \pm 30 $\mu\text{g}/24 \text{ hours}$

Total catecholamines (free and conjugated): 10 - 270 $\mu\text{g}/24 \text{ hours}$

NOTES

The quantity of the supplied reagent is enough to perform the following:
40 samples with related blanks, 8 Epinephrine standards with related blanks, 8 Norepinephrine standards with related blanks.

REFERENCES

1. T.G. Rosano, T.A. Swift et L.W. Hayes, "Clin. Chem.", 37 (10), 1854-1867 (1991)
2. E. Comoy et C. Bohuon, Clin. Chem. Acta , 30, 191-205 (1970)



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